

# Mutations Seen in Families with Factor X Deficiency Composed of Only Heterozygous Patients who Present a Bleeding Tendency

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## ABSTRACT

**Objective:** The objective of the study was to ascertain the mutations prevalent in families with patients with heterozygous Factor X (FX) deficiency and a bleeding tendency. **Patients and Methods:** Eight families with proven heterozygous FX deficiency were investigated. Two of these families were studied by the authors, whereas six were gathered from the literature. All families were composed only by heterozygotes or normal subject. No homozygotes were present. FX activity was evaluated according to the extrinsic (tissue thromboplastin), intrinsic (cephalin), and Russell viper venom-dependent assay. FX antigen was assayed by an ELISA method. Molecular analysis was carried out by the Sanger method. **Results:** FX activity was about 50% of normal regardless of the assay used. FX antigen was instead normal. The two home investigated families showed the Pro343Ser and the Arg405Gly mutation. The mutations reported in the other families were Asp282Asn, Gla25Lys, Gly366Ser, Arg114Gly, Arg405Gly, and Val196Met. Five of these mutation involved exon 8 of the catalytic domain. The remaining three involved exons 2, 5, or 6, respectively. **Conclusions:** There is no association between a specific mutation and the bleeding tendency. Bleeding in heterozygous FX deficiency correlates with FX activity level rather than with mutations.

**Key words:** Bleeding, Factor VII, heterozygotes

## INTRODUCTION

Heterozygous patients for rare bleeding disorders have usually a factor activity level of 40–60% of normal. Heterozygotes for Factor II (FII) and FX have been demonstrated to show a mild bleeding tendency as compared to unaffected family members. This mild tendency is characterized by easy bruising, epistaxis, menorrhagia, bleeding after tooth extraction, delivery, or surgical procedures.<sup>[1,2]</sup> On the contrary, heterozygotes for FVII defect have been demonstrated in a long-range prospective study to have occasional bleeding similar to that observed in unaffected family members.<sup>[3]</sup>

The clinical status of heterozygotes for FV and FXI deficiencies is still unknown.<sup>[4,5]</sup>

Only a minority (about 25%) of patients heterozygous for FII or FX deficiency present a mild bleeding tendency. The rest of patients remain asymptomatic. It is not known yet whether there are mutations in the gene of these clotting factors that are more frequently associated with bleeding or not.

The purpose of the present study is to report on two families with mildly symptomatic heterozygous FX deficiency, one from Italy (FX Friuli) and the other from Argentina and to compare the mutations found in these

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families with those seen in other similar families reported in the literature.

## PATIENTS AND METHODS

The first home discovered family was a family with FX Friuli that had been first studied by us in 1983 and was subsequently restudied by means of molecular biology techniques. In this family, there were four heterozygotes and one of them was mildly symptomatic.<sup>[6]</sup>

The second family with heterozygous FX deficiency has been identified in Cordoba, Argentina. The family tree is reported in Figure 1. The proposita and three of her relatives presented a mild bleeding tendency (easy bruising, epistaxis, bleeding after tooth extraction, and menorrhagia). The parents were not consanguineous, had a Spanish-Argentinean background, and were asymptomatic. Interestingly, the mother at the age of 22 years had pulmonary embolism during hormonal replacement therapy. She had no bleeding tendency. A brother of the proposita died at the age of 20 years during plastic surgery of the biliary tract. A sister of the proposita died at the age of 9 years for biliary cirrhosis. A son of the proposita died in infancy due to an infection.

Clotting tests were carried out in Cordoba.

When needed, assays were repeated in Padua on fresh frozen plasma as previously reported.<sup>[7,8]</sup>

FX activity was carried out using tissue thromboplastin, activated partial thromboplastin, and a mixture of Russell viper venom and cephalin. FX antigen was evaluated by Asserachrom FX (Stago Laboratories, Asniers, France).

Genetic analysis was carried out as previously reported.<sup>[7]</sup> Briefly, genomic DNA was prepared from leukocytes by standard procedures. Amplification of exon 1 through exon 8 and respective splice junctions of the FX gene was performed using oligonucleotide primer kindly supplied by Dr. James H. (Tyler, TX, USA) and other acquired from Invitrogen (Carlsbad, CA, USA).

Sequencing was performed according to the Sanger method<sup>[9]</sup> on an ABI Prism 310 DNA Sequencer (Applied Biosystems, Foster city, CA, USA) using ABI Prism big dye terminator cycle sequencing reaction kit with AmpliTaq DNA Polymerase FS (Applied Biosystems). Forward and reverse primer were the same as those used for polymerase chain reaction amplifications. The sequencing analysis 33 computer program (Applied Biosystems) supplied the sequencing data.

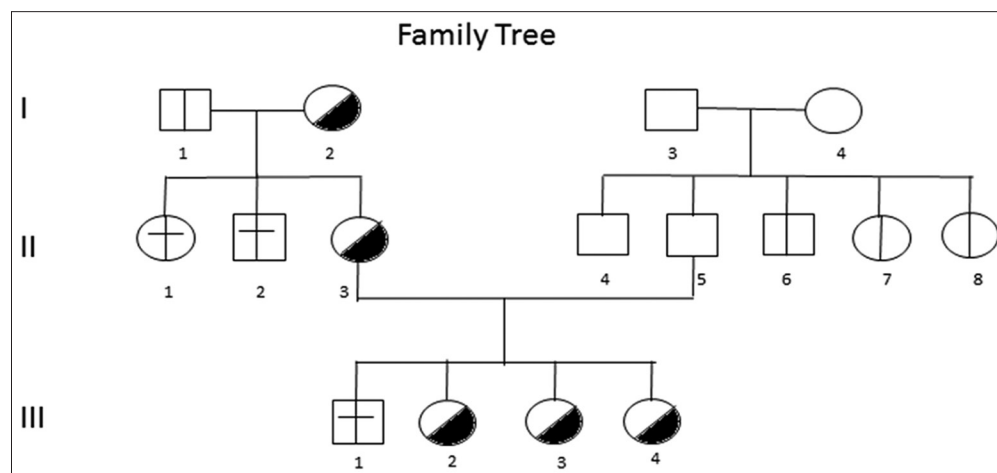
Results were compared with normal FX sequence as reported in the GenBank database.

Other families with only heterozygous FX deficiency were gathered from two sources: (1) Two time-unlimited PubMed searches were carried out between January 2007 and January 2017. Several key words including the Mesh items supplied by PubMed were used. Side tables were also examined whenever available (2) from personal files of patients who belonged to families which were composed only by heterozygotes.

Families with only heterozygotes but without molecular studies were excluded. Similarly, families with compound heterozygosity were also excluded.

FX assay as obtained through the extrinsic, intrinsic, or Russell viper venom assays was recorded.

FX antigen was also evaluated whenever available.



**Figure 1:** Proposita and three relatives were slightly symptomatic; ● = Heterozygotes for mutation; ⊕ or ⊞ deceased; □ or ○ normal; || or ⊖ not available for study;

The bleeding symptoms presented by the patients were listed, and the ratio between symptomatic or asymptomatic patients was evaluated.

The presence of associated potential bleeding risk was noted whenever available.

## RESULTS

The proband of the first home investigated family (FX Friuli) had FX activity level around 50% of normal, regardless of the method, whereas FX antigen was normal. He showed a mild bleeding tendency characterized by easy bruising, epistaxis, and bleeding after tooth extraction.

The probanda of the Argentinean family showed a mild bleeding tendency and had slight prolonged prothrombin time (PT) and partial thromboplastin time. FX level was about 50% of normal regardless of the activating system used extrinsic, intrinsic, or Russell viper venom FX antigen was normal. Three relatives of the probanda were also heterozygous and slightly symptomatic. The mother of the probanda showed no bleeding tendency despite being similarly affected. All other clotting factors were within normal limits. The family tree is reported in Figure 1. The other members of the family investigated had normal coagulation assays and had no bleeding tendency.

Molecular biology studies revealed the existence of a heterozygous missense mutation (Arg405Gly) in exon 8 [Figure 2].

A homozygous synonymous polymorphism (Thr224=) was also present in exon 7.

The synonymous mutation was present in the probanda and other affected carriers of the Arg405Gly mutation but also in normal subjects of the family.

The mutation in the family with heterozygous FX Friuli was already known to be Pro343Ser.<sup>[6,8]</sup>

The literature search for families with heterozygous FX deficiency allowed the identification of six other families which met the inclusion criteria. The main features of these families are reported in Table 1. The mutations observed in these families were located in exon 8 in five instances: Pro343Ser, Arg282Asn, Gly366Ser, Arg405Gly (twice), and exons 2, 5, and 6, namely, Gla25Lys, Arg114Gly, and Val196Met (one each).<sup>[10-15]</sup>

Three families were Type I defects (concomitant decrease of FX activity and FX antigen), whereas the remaining five were Type 2 (reduced FX activity, normal or near normal FX antigen) [Table 1].

## DISCUSSION

Homozygous or compound heterozygous FX deficiency is always symptomatic. The bleeding tendency may be variable, but it is often severe with brain hemorrhage, hematomas, and hemarthrosis.<sup>[16-18]</sup>

Heterozygotes for FX defects may present a mild bleeding tendency, particularly after tooth extraction, tonsillectomy, and other surgical procedures.<sup>[1,19]</sup>

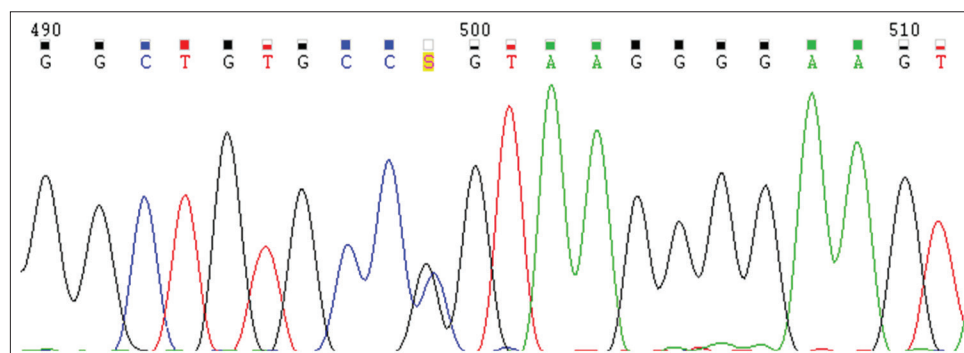
Easy bruising, epistaxis, and menorrhagia may also be present.

An analysis of the mutations found in families with only heterozygotes supplies a few useful indications.

The mutation Arg405Gly in exon 8 has been found in two families (FX Taunton) and the family from Argentina (second personal observation).

This mutation probably causes a conformational change that lowers the catalytic activity while the protein binding sites are conserved.

The mutation seen in FX Stockton (Asp282Asn) is also in exon 8.



**Figure 2:** Sequencing profile of the probanda showing the heterozygous mutation Arg405Gly in exon 8

**Table 1:** Main features of the probands belonging to families with proven heterozygous FX defects. The bleeding manifestations reported refer to the probands

Author (year)	Age and sex	Eponymous	FX activity %	FK antigen %	Bleeding	Replacement therapy	Mutation(exon)	Comments
Girolami <i>et al.</i> (1983)	27, F	FX Friuli	50 51 100	105	Easy bruising, bleeding after tooth extraction	None	Pro343Ser (8)	Three other family members similarly affected but asymptomatic
Messier <i>et al.</i>	n.r., F	FX Stockton	n.r. n.r. 43	101	Mild easy bruising, epistaxis, bleeding after tooth extraction, menorrhagia	None	Asp282Asn (8)	13 family members had mild FX deficiency. Concomitant mild FX deficiency in proposita and in other four family members. Combined FX+FX deficiency not ruled out
Miyata <i>et al.</i>	75, F	FX Nagoya 2	34 n.r. n.r.	80	Epistaxis	None	Gly366Ser (8)	
Nöbauer-Huhmann <i>et al.</i>	25, M	FX Frankfurt I	56 55 57	55	After tonsillectomy	None	Glu24Lys (2)	
Morishita <i>et al.</i> (2001)	70, M	FX Kanazawa	45 61 45	50	Moderate (post-polypectomy)	None	Gly114Arg (5)	A sister and two sons normal
Deam <i>et al.</i>	25, F	FX Taunton	44 45 49	78	Menorrhagia	None	Arg405Gly (8)	The proposita and four additional family members similarly affected, variably asymptomatic (menorrhagia, bleeding after tooth extraction)
Shinohara <i>et al.</i>	20, M	FX Hofu	51 n.r. n.r.	100	None	None	Val196Met (6)	Father probably affected but unavailable. Mother and brother normal
Present family	F	FX Cordoba (FX Taunton)	53 49 50	86	Easy bruising, menorrhagia	None	Arg405Gly (8)	Three family members similarly affected and symptomatic

N.r.: Not reported

This mutation causes also a decreased catalytic activity, but the protein level is also normal. The bleeding manifestation seen in the heterozygotes for FX Stockton need a word of caution since a concomitant reduction of FV activity is often present. The finding has been interpreted as the result of a competition between the retained abnormal FX protein and the wild-type FX in the binding with FV. However, no genetic study has been done on FV; therefore, the Stockton defect could be a case of combined FX and FV deficiency. That this is so suggested by the observation that the bleeding diathesis in these patients was more severe than usually that encountered in other heterozygotes for FX deficiency.

The bleeding was probably the result of two defects (FX+FV).

This interpretation is indirectly confirmed by the observation that in the new family from Argentina which shows the same mutation as that seen in FX Taunton, namely, Arg405Gly, FX antigen was normal, but FV activity was also found to be normal.<sup>[14]</sup>

The bleeding manifestation in the FX Friuli family (Pro343Ser) was also mild and limited to easy bruising and bleeding after tooth extraction in only one of the four heterozygotes present in the family.<sup>[9]</sup>

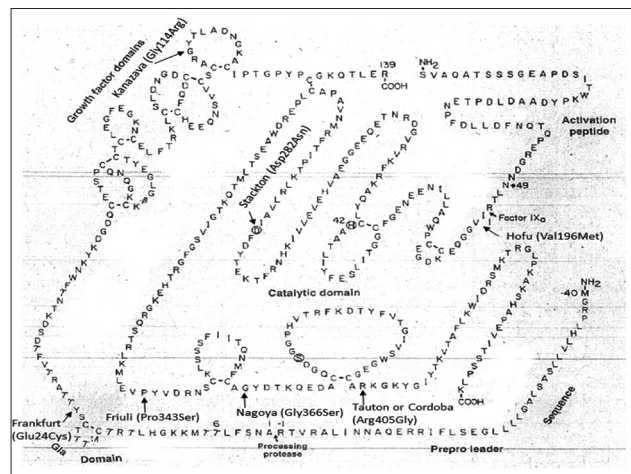
The identity between FX Taunton and FX Cordoba is justified not only by the missense mutation and by the clotting and antigenic results but also by the presence of a synonymous heterozygous mutation involving the polymorphism coding for Thr224 = in exon 7.<sup>[15]</sup> However, there is no linkage between this synonymous polymorphism in exon 7 and the missense mutation in exon 8 since the same polymorphism has been detected even in family normal members, namely, persons without the FX deficiency.

The other families showed different mutations, namely, Gly366Ser (FX Nagoya 2), Glu24Lys (FX Frankfurt I), Arg114Gly (FX Kanazawa), and Val196Met (FX Hofu).

Five mutations involved exon 8 and, one each, involved exons 2, 5, or 6. This indicates that the majority of families showed mutations in the catalytic domain [Figure 3].<sup>[20]</sup>

On the basis of this study, it is likely that there is no mutation, particularly associated with bleeding. Bleeding correlates with FX activity level, regardless of the underlying mutation.<sup>[1,19]</sup>

Altogether the bleeding manifestations were mild and similar to those presented by FX Friuli and FX Taunton [Table 2].



**Figure 3:** Schematic representation of FX molecule as proposed by Greenberg and Davie<sup>[20]</sup>

Table 2: Prevalence of bleeding in the families with heterozygotes for FX deficiency					
Author (year)	Eponym	N° of heterozygotes in the family	N° of patients with bleeding	Mutation (exon)	Comments
Girolami <i>et al.</i> (1983)	Friuli	4	1	Pro343Ser (8)	
Messier <i>et al.</i>	Stockton	14	4	Asp282Asn (8)	Cases with concomitant FV deficiency excluded
Nöbauer-Huhmann <i>et al.</i>	Frankfurt	3	0	Gla25Lys (2)	
Miyata <i>et al.</i>	Nagoya 2	1	1	Gly366Ser (8)	
Morishita <i>et al.</i> (2001)	Kanazawa	1	1	Arg114Gly (5)	Sister and two sons normal
Deam <i>et al.</i>	Taunton	8	5	Arg405Gly (8)	
Shinohare <i>et al.</i>	Hofu	1	1	Val196Met (6)	
Present family	Cordoba (Taunton)	5	4	Arg405Gly (8)	
Gran total	/	37	17 (45.9%)	/	

The mean prevalence of bleeding in this group of families with heterozygous FX deficiency was 45.9%. This value is similar to that observed in a long-range, prospective follow-up study.<sup>[1]</sup> This study on the mutations seen in patients with heterozygous FX deficiency confirms the fact that these patients may have a potential, even though mild, bleeding diathesis.

Since bleeding occurs mainly after surgical procedures including tooth extractions, other surgical procedures, or deliveries, it is important that the caring physicians be informed of the possible complication. This is often overlooked because the PT may be borderline or only slightly prolonged and no specific FX assay is available.

Patients belonging to families with FX deficiency, even if asymptomatic, should be carefully investigated and followed before and during surgical procedures or at delivery.

The authors declare that they have no conflict of interest.

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